PCT WORLD INTELLECTUAL PROPE International Bu

INTERNATIONAL APPLICATION PUBLISHED UNDER WO 9605353A1

(51) International Patent Classification 6 : D06L 1/14, C12N 9/42, 15/56 — A1

(11) International Publication Number: WO 96/05353

(43) International Publication Date: 22 February 1996 (22.02.96)

(21) International Application Number: PCT/DK95/00328

(22) International Filing Date: 14 August 1995 (14.08.95)

(30) Priority Data:
0937/94 15 August 1994 (15.08.94) DK

(71) Applicant *(for all designated States except US)*: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: LUND, Henrik [DK/DK]; Novo Nordisk A/S, Novo Allé, DK-2880 Bagsværd (DK).

(74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: A METHOD FOR DESIZING CELLULOSE-CONTAINING FABRIC

(57) Abstract

Cellulose-containing fabrics or textile may be desized without essentially damaging the fabric or textile by subjecting the fabric or textile to a treatment with a certain type of cellulolytic enzyme, e.g. a cellulolytic enzyme having an activity on carboxymethylcellulose (CMC) and a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} of at least 0.01 s^{-1} and preferably being of microbial origin, more preferably being obtainable by or derived from a strain of *Humicola, Trichoderma, Myceliophthora, Penicillium, Irpex, Aspergillus, Scytalidium* or *Fusarium*.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

A METHOD FOR DESIZING CELLULOSE-CONTAINING FABRIC

The present invention relates to a method for desizing cellulose-containing textiles or fabric, and an enzyme composition for use in the method.

BACKGROUND OF THE INVENTION

During the weaving of textiles, the threads are exposed to considerable mechanical strain. In order to prevent breaking, they are usually reinforced by coating (sizing) with a gelatinous substance (size). The most common sizing agent is starch in native or modified form, yet other polymeric compounds such as polyvinylalcohol (PVA), polyvinylpyrrolidone (PVP), polyacrylic acid (PAA) or derivatives of cellulose (e.g. carboxymethylcellulose(CMC), hydroxyethylcellulose, hydroxypropylcellulose or methylcellulose) may also be abundant in the size. A small amount of e.g. fats and oils may be added to the size, also, with the aim of lubricating the surface.

As a consequence of the sizing, the threads are not able to absorb water or finishing agents or compositions (bleaching, dyeing, crease-proofing etc.) to a sufficient degree. A uniform and durable finishing can thus be obtained only after removal of the size from the fabric, the so-called desizing.

In cases where the size comprises a significant amount of carboxymethylcellulose (CMC) or other cellulose-derivatives, the desizing treatment may be carried out with a cellulolytic enzyme, either alone or in combination with other substances, optionally in combination with other enzymes, e.g. starch-degrading enzymes such as amylases. However, such treatment with cellulolytic enzymes may, in case of sized fabric or textile containing cellulose or cellulose-derivatives, not only fully or partly degrade the size but also degrade the textile or fabric, thus resulting

in a desized fabric or textile having a strength loss and/or a weight loss as compared to the strength or weight of the unsized textile or fabric.

The object of the present invention is to provide a method for desizing cellulose-containing textile or fabric essentially without damaging the fabric, i.e. without inducing to the fabric a strength loss or a weight loss or both.

SUMMARY OF THE INVENTION

Surprisingly, it has been found that it is possible to desize cellulose-containing fabric or textile without essentially damaging the fabric or textile by subjecting the fabric or textile to a treatment with a certain type of cellulolytic enzyme.

Accordingly, the present invention provides a method for desizing cellulose-containing fabric or textile, wherein the fabric or textile is treated with a cellulolytic enzyme having an activity on carboxymethylcellulose (CMC) and a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} of at least 0.01 per sec.

In another aspect, there is provided a composition for use in the method of the invention, the composition comprising a cellulolytic enzyme having an activity on carboxymethylcellulose (CMC) and a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} of at least 0.01 per sec. It is to be understood that the composition should not comprise any cellulase component capable of inducing fabric damage.

By using the method of the invention may be obtained desized cellulose-containing fabric or textile which exhibits essentially no strength loss or weight loss as compared to the unsized fabric or textile.

DETAILED DESCRIPTION OF THE INVENTION

The term "desizing" is intended to be understood in a conventional manner, i.e. the removal of size from the fabric.

In the present specification and claims, the terms "cellulolytic enzyme", "cellulase" and "cellulase component" are intended to mean an enzyme that hydrolyses cellulose. The cellulolytic enzyme, cellulase or cellulase component may be a component occurring in a cellulase system produced by a given microorganism, such a cellulase system mostly comprising several different cellulase enzyme components including those usually identified as e.g. cellobiohydrolases, exo-cellobiohydrolases, endo-β-1,4-glucanases, β-glucosidases. Alternatively, the cellulase component may be a single component, i.e. a component essentially free of other cellulase components usually occurring in a cellulase system produced by a given microorganism, the single component being a recombinant component, i.e. produced by cloning of a DNA sequence encoding the single component and subsequent cell transformed with the DNA sequence and expressed in a host. The host is preferably a heterologous host, but the host may under certain conditions also be the homologous host.

The native or unmodified cellulase or cellulase component may be derived from microorganisms which are known to be capable of producing cellulolytic enzymes, e.g. species of *Humicola, Thermomyces, Bacillus, Trichoderma, Fusarium, Myceliophthora, Phanerochaete, Irpex, Scytalidium, Schizophyllum, Penicillium, Aspergillus*, and *Geotricum*. The derived component may be either homologous or heterologous component. Preferably, the component is homologous. However, a heterologous component which is immunologically reactive with an antibody raised against a highly purified cellulase component and which is derived from a specific microorganism is also preferred.

It is preferred that a useful cellulase to be used in the present process is of microbial origin. In a preferred embodiment of the invention, the cellulolytic enzyme is obtainable by or derived from a strain selected from the group consisting of *Humicola, Trichoderma, Myceliophthora, Penicillium, Irpex, Aspergillus, Scytalidium* or *Fusarium*. More preferably, the enzyme is obtainable by or derivable from a strain of *Humicola insolens, Fusarium oxysporum* or *Trichoderma reesei*.

In the present context the term "derivable" or "derived from" is intended not only to indicate a cellulase produced by a strain of the organism in question, but also a cellulase encoded by a DNA sequence isolated from such strain and produced in a host organism transformed with said DNA sequence. Furthermore, the term is intended to indicate a cellulase which is encoded by a DNA sequence of synthetic and/or cDNA origin and which has the identifying characteristics of the cellulase in question.

Preferably, the cellulolytic enzyme to be used in the method of the invention is a recombinant cellulase, i.e. a cellulase essentially free from other proteins or cellulase proteins. A recombinant cellulase component may be cloned and expressed according to standard techniques conventional to the skilled person.

Advantageously, a parent cellulase of fungal origin may be used, e.g. a cellulase derivable from a strain of the fungal genus *Humicola* or *Fusarium*. For instance, the parent cellulase may be derivable from a strain of the fungal species *H. insolens* or F. oxysporum. Many of these cellulases are all well characterized and their entire amino acid sequence are described.

In a preferred embodiment the parent cellulase is selected from the group consisting of a *H. insolens, F. oxysporum* and

Trichoderma reesei cellulase, or is a functional analogue of any of said parent cellulases which

i) comprises an amino acid sequence being at least 60% homologous with the amino acid sequence of the parent cellulase,

ii) reacts with an antibody raised against the parent cellulase, and/or

iii) is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding the parent cellulase.

Property i) of the analogue is intended to indicate the degree of identity between the analogue and the parent cellulase indicating a derivation of the first sequence from the second. In particular, a polypeptide is considered to be homologous to the parent cellulase if a comparison of the respective amino acid sequences reveals an identity of greater than about 60%, such as above 70%, 80%, 85%, 90% or even 95%. Sequence comparisons can be performed via known algorithms, such as the one described by Lipman and Pearson (1985).

The homologous cellulase may be a genetically engineered cellulase, e.g. prepared in order to improve one or more properties such as thermostability, acid/alkaline stability, temperature or pH optimum and the like.

The additional properties ii) and iii) of the analogue of the parent cellulase may be determined as follows:

Property ii), i.e. the immunological cross reactivity, may be assayed using an antibody raised against or reactive with at least one epitope of the parent cellulase. The antibody, which may either be monoclonal or polyclonal, may be produced by methods known in the art, e.g. as described by

Hudson et al., 1989. The immunological cross-reactivity may be determined using assays known in the art, examples of which are Western Blotting or radial immunodiffusion assay, e.g. as described by Hudson et al., 1989.

The oligonucleotide probe used in the characterization of the analogue in accordance with property iii) defined above, may suitably be prepared on the basis of the full or partial nucleotide or amino acid sequence of the parent cellulase. The hybridization may be carried out under any suitable conditions allowing the DNA sequences to hybridize. For instance, such conditions are hybridization under specified conditions, e.g. involving presoaking in 5xSSC and prehybridizing for 1h at ~40°C in a solution of 20% formamide, 5xDenhardt's solution, 50mM sodium phosphate, pH 6.8, and 50µg of denatured sonicated calf thymus DNA, followed by hybridization in the same solution supplemented with 100µM ATP for 18h at ~40°C, or other methods described by e.g. Sambrook et al., 1989.

Examples of cellulolytic enzyme useful according to the invention are:

An endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~50kD endoglucanase derived from *Humicola insolens*, DSM 1800, or which is a homologue or derivative of the ~50kD endoglucanase exhibiting cellulase activity. A preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No. WO91/17244, Fig. 14A-E, which is shown in the appended SEQ ID NO:2, or a variant of said endoglucanase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous with said sequence. Below, the endoglucanase component is referred to as EG I; and

an endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~50kD (apparent molecular weight, the amino acid composition corresponds to 45kD with 2n glycosylation sites) endoglucanase derived from *Fusarium oxysporum*, DSM 2672, or which is a homologue or derivative of the ~50kD endoglucanase exhibiting cellulase activity. A preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No. WO91/17244, Fig. 13, which is shown in the appended SEQ ID NO:3, or a variant of said endoglucanase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous with said sequence. Below, the endoglucanase component is referred to as EG I-F. The EG I-F cellulase component is producible by *Aspergillus oryzae* after transformation with a plasmid containing the DNA sequence corresponding to the amino acid sequence of the appended SEQ ID NO:3 and using the conventional Taka promoter and AMG terminator. The EG I-F may be purified to homogeneity using cationic chromatography and has a pI >9. The calculated pI is 9 based on the amino acid composition using the PHKa values from *Adv. Protein Chem. 17, p. 69-165 (1962)(C. Tanford)*. The molar extinction coefficient is calculated to be 58180; and

any of the cellulases disclosed in the published European Patent Application No. EP-A2-271 004, the cellulase having a non-degrading index (NDI) of not less than 500 and being an alkalophilic cellulase having an optimum pH not less than 7 or whose relative activity at a pH of not less than 8 is 50% or over of the activity under optimum conditions when carboxy methyl cellulose (CMC) is used as a substrate; the cellulase preferably being selected from the group consisting of alkaline cellulase K (produced by Bacillus sp. KSM-635, FERM BP 1485); alkaline cellulase K-534 (produced by Bacillus sp. KSM-534, FERM BP 1508); alkaline cellulase K-539 (produced by Bacillus sp. KSM-539, FERM BP 1509); alka-

line cellulase K-577 (produced by Bacillus sp. KSM-577, FERM BP 1510); alkaline cellulase K-521 (produced by Bacillus sp. KSM-521, FERM BP 1507); alkaline cellulase K-580 (produced by Bacillus sp. KSM-580, FERM BP 1511); alkaline cellulase K-588 (produced by Bacillus sp. KSM-588, FERM BP 1513); alkaline cellulase K-597 (produced by Bacillus sp. KSM-597, FERM BP 1514); alkaline cellulase K-522 (produced by Bacillus sp. KSM-522, FERM BP 1512); CMCase I, CMCase II (both produced by Bacillus sp. KSM-635, FERM BP 1485); alkaline cellulase E-II and alkaline cellulase E-III (both produced by Bacillus sp. KSM-522, FERM BP 1512).

The terms "cellulose-containing textile/fabric" and "cellulosic textile/fabric" are intended to indicate any type of fabric, in particular woven fabric, prepared from a cellulose-containing material, containing cellulose or cellulose derivatives, e.g. from wood pulp, and cotton. The main part of the cellulose or cellulose derivatives present on the fabric is normally size with which the yarns, normally warp yarns, have been coated prior to weaving. In the present context, the term "fabric" is also intended to include garments and other types of processed fabrics. Examples of cellulosic fabric is cotton, viscose (rayon); lyocell; all blends of viscose, cotton or lyocell with other fibers such as polyester; viscose/cotton blends, lyocell/cotton blends, viscose/wool blends, lyocell/wool blends, cotton/wool blends; flax (linen), ramie and other fabrics based on cellulose fibers, including all blends of cellulosic fibers with other fibers such as wool, polyamide, acrylic and polyester fibers, e.g. viscose/cotton/polyester blends, wool/cotton/polyester blends, flax/cotton blends etc.

Process conditions

It will be understood that the method of the invention may be performed in accordance with any suitable desizing process known in the art, e.g. as described by Olson, E.S.

(1983), and Peter, M. and Rouette H.K. (1988). Thus, the process conditions to be used in performing the present invention may be selected so as to match a particular equipment or a particular type of process which it is desirable to use. Preferred examples of process types to be used in connection with the present invention include Jigger/Winch, Pad-Roll and Pad-Steam types. These types are dealt with in further detail below.

The method of the invention may be carried out as a batch, semi-continuous or continuous process. As an example the method may comprise the following steps:

(a) Impregnating the fabric in a desizing bath containing (as a minimum) a cellulase having an activity on carboxymethyl-cellulose (CMC) and a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} of at least 0.01 per sec. followed by squeezing out excessive liquid so as to maintain the quantity of liquor necessary for the reaction to be carried out (normally between 60 and 120% of the weight of the dry fabric),

(b) subjecting the impregnated fabric to steaming so as to bring the fabric to the desired reaction temperature, generally between 20° and 120°C, and

(c) holding by rolling up or pleating the cloth in a J-Box, U-Box, carpet machine or the like for a sufficient period of time (normally between a few minutes and several hours) to allow the desizing to occur.

Prior to the treatment to be performed the useful cellulase may conveniently be mixed with other components which are conventionally used in the desizing process. Examples of such components are other enzymes, preferably such commercially available amylases which conventionally are used for desizing such as the microbial amylases, especially amylases

producible by *Bacillus*, e.g. *B. licheniformis, B. amyloliquefaciens, B. subtilis, B. stearothermophilus*, or *Aspergillus*; or mutants thereof, e.g. as described in WO 91/00353 or WO 91/16423. Examples of commercial amylases for desizing are Aquazym®, Aquazym Ultra, Dezyme®, Thermozyme™ and Termamyl® from Novo Nordisk A/S. Further preferred amylases are the oxidation-stable α-amylase mutants disclosed in International Patent Application PCT/DK94/00371, which is hereby incorporated by reference.

Thus, in the composition of the present invention it may be advantageous to incorporate an α-amylase having improved ocidation stability so as to make the composition useful in a combined desizing and bleaching process (performed in a single operation), e.g. a process using sodium chlorite in combination with a strong base, a surfactant, an activator, an amylolytic enzyme and optionally a cellulolytic enzyme; or a process using sodium tetraborate dexahydrate as a buffer in a bath containing hydrogen peroxide, a sequestering agent, a surfactant, an amylolytic enzyme and optionally a cellulolytic enzyme.

Further components required for the process to be performed may be added separately. Examples of such components include a stabilizer and a wetting agent. The stabilizer may be an agent stabilizing the cellulolytic enzyme.

The wetting agent serves to improve the wettability of the fibre whereby a rapid and even desizing may be obtained. The wetting agent is preferably of an oxidation stable type.

In a preferred embodiment of the process of the invention, the useful cellulase is used in an amount exceeding 1 g/l, preferably in an amount of 1-20 g/l, such as 1-10 g/l, 1-5 g/l or 1-3 g/l, corresponding to a cellulase activity in the range of between 10 and 5000 ECU per litre of desizing liquor, preferably between 50 and 500 ECU per litre of

desizing liquor. It will be understood that the amount of cellulase to be used depend on the formulation of the cellulase product in question.

Irrespective of the particular type of process to be used for the desizing of the invention, the method of the invention is normally performed at a temperature in the range of 30-100°C, such as 35-60°C, and a pH in the range of 3-11, preferably 7-9. However, the actual process conditions may vary widely within these ranges as will be apparent from the following disclosure.

Preferred examples of the process conditions to be used in connection with the present invention include:

A batch type process, e.g. of the Jigger/Winch type, using 1-5 g/l of a useful cellulase, and 0.25-5 g/l of a wetting agent, e.g. the commercial product Arbyl R available from Grünau, Germany, the process being performed at a pH in the range of 7-9 (obtained by addition of NaOH) and a temperature in the range of 40-55°C, typically for 1-2 hours.

A semi-continuous process, e.g. of the Pad-Roll type, using 1-5 g/l of a useful cellulase, 0.25-5 g/l of a wetting agent, e.g. Arbyl R, the process being performed at a pH in the range of 7-9 (possibly obtained by addition of NaOH) and a temperature in the range of 30-50°C, typically for 12-24 hours.

It will be understood that the method may be performed in any equipment sufficiently tolerant towards the conditions of the method.

Furthermore, it will be evident that in addition to the k_{cat} restriction of the cellulase to be used in the method of the invention this cellulase should preferably be one which is active at a pH of above 3, such as above about pH 7. Prefer-

ably the cellulase has a high activity in the pH range of 7-9.

The method of the invention may be used prior to or after another desizing treatment step which may supplement the cellulase treatment, the supplementing treatment preferably being an enzymatic desizing treatment with an amylase.

Composition of the invention

Although the useful cellulase may be added as such it is preferred that it is formulated into a suitable desizing composition preferably further comprising other desizing enzymes, e.g. amylases as described above.

The desizing composition of the invention may be a mixture of each enzyme in the form of a granulate, preferably a non-dusting granulate, a liquid, in particular a stabilized liquid, a slurry, or in a protected form. Dust free granulates may be produced, e.g. as disclosed in US 4,106,991 and US 4,661,452 (both to Novo Nordisk A/S) and may optionally be coated by methods known in the art.

Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as e.g. propylene glycol, a sugar or sugar alcohol or acetic acid, according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238 216.

In principle the composition of the invention comprising a cellulolytic enzyme having an activity on carboxymethylcellulose (CMC) and a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} of at least 0.01 per sec. and optionally an amylase may contain any other agent to be used in the combined process of the invention. However, it is preferred that the composition is free from a bleaching

agent and other highly oxidizing agents.

The composition of the invention may comprise one or more further components selected from the group consisting of wetting agents, dispersing agents, sequestering agents and emulsifying agents. Examples of suitable wetting agents are disclosed above. The emulsifying agent serves to emulsify hydrophobic impurities present on the fabric. The dispersing agent serves to prevent that extracted impurities redeposit on the fabric. The sequestering agent serve to remove ions such as Ca, Mg and Fe, which may have a negative impact on the process and preferred examples include caustic soda (sodium hydroxide) and soda ash (sodium carbonate).

Determination of activity of cellulase on cellotriose

The cellulase activity on cellotriose, in terms of k_{cat} (s^{-1}), may be determined by a coupled assay:

Cellotriose → Glucose + Cellobiose (cat.: cellulase)

Glucose + O_2 + H_2O → Gluconate + H_2O_2 (cat.: Glucoseoxidase)

H_2O_2 + $ABTS^R$ → $ABTS^{Ox}$ (cat.: Peroxidase)

which is followed spectrophotometrically at 418 nm (maximum absorbance of $ABTS^{Ox}$ at 418 nm).

Method:

The GOD-Perid Test Kit (available from Boehringer Mannheim, art. 124 036) was used. The buffer-enzyme solution in the test kit was dissolved in 500 ml milli Q water. pH of the solution was adjusted to 8.5 (NaOH).

80 mg of ABTS^R (available from Boehringer Mannheim, art. 756 407) was dissolved in 10 ml GOD-Perid corresponding to a total concentration of ABTS^R of 10 mg/ml.

A substrate stock solution of 5 mmole (2.52 mg/ml) of cellotriose (available from Merck art. 24741) in water was prepared. Diluted solutions in water corresponding to 1000 µmole, 500 µmole, 376 µmole, 250 µmole, 100µmole and 60 µmole were prepared.

The reaction mixture was prepared by mixing 1 part of substrate solution with 1 part of GOD-Perid.

A solution of the cellulase enzyme to be determined in a concentration of 1.0 - 3.0 µmole was prepared.

50 µl of enzyme solution and 450 µl of reaction mixture were mixed.

The measurements were carried out on a HP 8452A Diode Array Spectrophotometer thermostated at 40°C, 1 cm cuvette, at a wavelength of 418 nm. The reaction was followed by measuring the oxidation of ABTS every 20 sec for 600 sec in total.

Calculations:

The cellulase activity on cellotriose, in terms of k_{cat} (s^{-1}), was calculated from a Lineweaver-Burk plot (a plot of 1/V versus 1/[S]): the slope and the intersection were determined by linear regression analysis.

The following constants were used for the calculations:

Cellulase: ϵ = 66,310 $M^{-1} \cdot cm^{-1}$

$ABTS^{Ox}$: ϵ = 0.0323 $\mu mole^{-1} \cdot cm^{-1}$

The following results were obtained from the assay:

Enzyme	k_{cat} (s^{-1})
EG I	1.5
EG I-F	5.5

The cellulolytic activity of endoglucanase is determined relative to an analytical standard and may be expressed in the unit ECU.

Cellulolytic enzymes hydrolyse CMC, thereby decreasing the viscosity of the incubation mixture. The resulting reduction in viscosity may be determined by a vibration viscosimeter (e.g. MIVI 3000 from Sofraser, France).

Determination of the cellulolytic activity, measured in terms of ECU, may be determined according to the analysis method AF 301.1 which is available from the Applicant upon request.

The ECU assay quantifies the amount of catalytic activity present in the sample by measuring the ability of the sample to reduce the viscosity of a solution of carboxy-methylcellulose (CMC). The assay is carried out at 40°C, pH 7.5 using a relative enzyme standard for reducing the viscosity of the CMC substrate.

The invention is further illustrated by the following non-limiting examples.

EXAMPLE 1

Incubation of cotton knitwear with *Humicola insolens* EG I and EG V in Launder-O-meter.

In order to illustrate the remarkably low activity of

Endoglucanase I (EG I) from *Humicola insolens* towards cotton fabrics a comparative study with the *Humicola insolens* Endoglucanase V (EG V, ~43kDa, disclosed in WO 91/17243) was conducted. Both cellulases have a considerable activity in terms of ECU and are thus capable of degrading carboxymethylcellulose and numerous other hydro-colloidal cellulose-derivatives which may be present in a size composition.

Desized 100% cotton knitwear was treated in a Launder-Ometer with the two different enzymes under the following conditions:

Enzyme	Dosage	Buffer
H. insolens EG I	5.0 ECU/ml	2 g/l KH_2PO_4/NaOH pH 7.3
H. insolens EG V	1.0 ECU/ml	2 g/l KH_2PO_4/NaOH pH 7.0

Fabric: Bleached interlock knitted 100% cotton (205g/m^2), 7g per beaker (2 swatches, each approx. 13cm x 13cm).

Liquor volume: 140 ml (LQR 1:20)

Incubation: 60 minutes at 55°C

To each Launder-O-meter beaker were added 20 steel balls (d = 14mm, w = 11g) in order to increase the mechanical effect on the fabric during the cellulase treatment.

The following results were obtained:

Enzyme	Dosage	Weight loss (%)
H. insolens EG I	5.0 ECU/ml	0.1
H. insolens EG V	1.0 ECU/ml	3.9

Weight loss is defined as follows:

Percentage weight loss (%) =
[1 - (weight after treatment)/(weight before treatment)]·100

The results demonstrate that the EG I cellulase gives essentially no weight loss on cotton as compared to the weight loss induced by the EG V cellulase.

EXAMPLE 2

This example illustrates that the use of an cellulolytic enzyme characterized by the features set forth in the claims can provide a measurable excess removal of CMC-size and produce a remarkable improvement of fabric handle/stiffness and, further, that the enzyme has a beneficial action on fabrics sized with compositions made up of mixtures of CMC and starch/starch-derivatives.

Size compositions made up of pure carboxymethylcellulose (CMC) or that are very rich in CMC will usually tend to be largely soluble in water, i.e. the main part of a CMC size may be dissolved rapidly just by contacting the sized fabric with an aquous solution. Still, a minor part of the CMC-size will stick strongly to the fabric and give the fabric a very stiff hand. This stiffness will make the fabric unsuitable for further finishing.

The fabric used in these experiments was a 100% cotton interlock jersey. The fabric had prior to the desizing

experiments been sized with either pure CMC (Blanose 7LFD available from Aqualon GmbH, Germany) or a mixed size made up of 1:1 (w/w) blend of CMC (Blanose 7LFD available from Aqualon GmbH, Germany) and carboxymethylated starch (CMS, Solvitose C5 available from Lamberti S.p.a., Italy).

The amount of size on the fabric was approximately 6.5% (on weigth of the fabric) for the CMC-size , and approximately 5.6% (on weight of the fabric) for the CMC/CMS-size.

The fabrics were cut into swatches of 12cm x 14cm (weigth of approximately 3.54 g/swatch without size).

The swatches were weighed after climatization and then incubated for 30 mins in 250 ml glass beakers containing 200 ml 2g/l K-phoshate buffer pH 7.0 at 50°C including enzyme according the table below:

Series	Size	Cellulase	Amylase
1	CMC	no	no
2	CMC	20 ECU/g fabric	no
3	CMC/CMS	no	100KNU/g fabric
4	CMC/CMS	20 ECU/g fabric	100KNU/g fabric

Cellulase: EG I from *H. insolens* (as in example 1).

Amylase: Aquazym 120L (activity: 120 KNU/g), bacterial amylase commercially available from Novo Nordisk A/S.

After incubation the swatches were oven-dried at 103°C for 60 min, climatized and then weighed in order to evaluate the size removal. Average data on size removal are given in the table below:

Series	avg g size/swatch	avg g size removed/swatch	% removed
1	0.23	0.21	91%
2	0.23	0.23	100%
3	0.20	0.15	75%
4	0.20	0.17	85%

As can be seen the cellulase do in both cases - with CMC-size and CMC/CMS-size, respectively - facilitate the size removal.

For the CMC-size a large removal is seen also when the swatches are incubated in buffer without cellulase, yet from the fabric handle it was obvious that the remaining CMC (about 20 mg/swatch) had a pronounced effect on the fabric stiffness.

To illustrate this effect a panel of 5 people experienced in sensory evaluation of fabrics were asked to rank swatches from each series on a scale from 1 to 4, where Note 1 was given to the stiffest fabrics and Note 4 to the softest (best desizing result).

The effects were so pronounced that all members of the test-panel gave the exact same ranking as reflected in the table below:

Series	Note (avg)
1	2
2	4
3	1
4	3

Comparing Series 1 vs Series 2 and Series 3 vs Series 4 reveal a significant reduction in fabric stiffness and accordingly better desizing, resulting from the treatment with cellulolytic enzyme.

REFERENCES CITED IN THE SPECIFICATION

Lipman and Pearson, Science 227, 1435 (1985);

Hudson, L., and Hay, F., Practical Immunology, Third edition (1989), Blackwell Scientific Publications;

Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989;

Olson, E.S. Textile Wet Processes, Vol. I, Noyes Publication, Park Ridge, New Jersey, USA (1983);

M. Peter und H.K Rouette, Grundlagen der Textilveredlung, Deutsche Fachverlag GmbH, Frankfurt am Main, Germany (1988);

SEQUENCE LISTING

INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 415 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Humicola insolens
(B) STRAIN: DSM 1800

SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Gln Lys Pro Gly Glu Thr Lys Glu Val His Pro Gln Leu Thr Thr Phe
1               5                  10                  15

Arg Cys Thr Lys Arg Gly Gly Cys Lys Pro Ala Thr Asn Phe Ile Val
            20                  25                  30

Leu Asp Ser Leu Ser His Pro Ile His Arg Ala Glu Gly Leu Gly Pro
        35                  40                  45

Gly Gly Cys Gly Asp Trp Gly Asn Pro Pro Pro Lys Asp Val Cys Pro
    50                  55                  60

Asp Val Glu Ser Cys Ala Lys Asn Cys Ile Met Glu Gly Ile Pro Asp
65                  70                  75                  80

Tyr Ser Gln Tyr Gly Val Thr Thr Asn Gly Thr Ser Leu Arg Leu Gln
                85                  90                  95

His Ile Leu Pro Asp Gly Arg Val Pro Ser Pro Arg Val Tyr Leu Leu
            100                 105                 110

Asp Lys Thr Lys Arg Arg Tyr Glu Met Leu His Leu Thr Gly Phe Glu
        115                 120                 125
```

Phe Thr Phe Asp Val Asp Ala Thr Lys Leu Pro Cys Gly Met Asn Ser
130 135 140

Ala Leu Tyr Leu Ser Glu Met His Pro Thr Gly Ala Lys Ser Lys Tyr
145 150 155 160

Asn Pro Gly Gly Ala Tyr Tyr Gly Thr Gly Tyr Cys Asp Ala Gln Cys
165 170 175

Phe Val Thr Pro Phe Ile Asn Gly Leu Gly Asn Ile Glu Gly Lys Gly
180 185 190

Ser Cys Cys Asn Glu Met Asp Ile Trp Glu Ala Asn Ser Arg Ala Ser
195 200 205

His Val Ala Pro His Thr Cys Asn Lys Lys Gly Leu Tyr Leu Cys Glu
210 215 220

Gly Glu Glu Cys Ala Phe Glu Gly Val Cys Asp Lys Asn Gly Cys Gly
225 230 235 240

Trp Asn Asn Tyr Arg Val Asn Val Thr Asp Tyr Tyr Gly Arg Gly Glu
245 250 255

Glu Phe Lys Val Asn Thr Leu Lys Pro Phe Thr Val Val Thr Gln Phe
260 265 270

Leu Ala Asn Arg Arg Gly Lys Leu Glu Lys Ile His Arg Phe Tyr Val
275 280 285

Gln Asp Gly Lys Val Ile Glu Ser Phe Tyr Thr Asn Lys Glu Gly Val
290 295 300

Pro Tyr Thr Asn Met Ile Asp Asp Glu Phe Cys Glu Ala Thr Gly Ser
305 310 315 320

Arg Lys Tyr Met Glu Leu Gly Ala Thr Gln Gly Met Gly Glu Ala Leu
325 330 335

Thr Arg Gly Met Val Leu Ala Met Ser Ile Trp Trp Asp Gln Gly Gly
340 345 350

Asn Met Glu Trp Leu Asp His Gly Glu Ala Gly Pro Cys Ala Lys Gly
355 360 365

Glu Gly Ala Pro Ser Asn Ile Val Gln Val Glu Pro Phe Pro Glu Val
370 375 380

Thr Tyr Thr Asn Leu Arg Trp Gly Glu Ile Gly Ser Thr Tyr Gln Glu
385 390 395 400

Val Gln Lys Pro Lys Pro Lys Pro Gly His Gly Pro Arg Ser Asp
405 410 415

INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 409 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Fusarium oxysporum
(B) STRAIN: DSM 2672

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gln Thr Pro Asp Lys Ala Lys Glu Gln His Pro Lys Leu Glu Thr Tyr
1 5 10 15

Arg Cys Thr Lys Ala Ser Gly Cys Lys Lys Gln Thr Asn Tyr Ile Val
20 25 30

Ala Asp Ala Gly Ile His Gly Ile Arg Arg Ser Ala Gly Cys Gly Asp
35 40 45

Trp Gly Gln Lys Pro Asn Ala Thr Ala Cys Pro Asp Glu Ala Ser Cys
50 55 60

Ala Lys Asn Cys Ile Leu Ser Gly Met Asp Ser Asn Ala Tyr Lys Asn
65 70 75 80

Ala Gly Ile Thr Thr Ser Gly Asn Lys Leu Arg Leu Gln Gln Leu Ile
85 90 95

Asn Asn Gln Leu Val Ser Pro Arg Val Tyr Leu Leu Glu Glu Asn Lys
100 105 110

Lys Lys Tyr Glu Met Leu His Leu Thr Gly Thr Glu Phe Ser Phe Asp
115 120 125

Val Glu Met Glu Lys Leu Pro Cys Gly Met Asn Gly Ala Leu Tyr Leu
130 135 140

Ser Glu Met Pro Gln Asp Gly Gly Lys Ser Thr Ser Arg Asn Ser Lys
145 150 155 160

Ala Gly Ala Tyr Tyr Gly Ala Gly Tyr Cys Asp Ala Gln Cys Tyr Val
165 170 175

Thr Pro Phe Ile Asn Gly Val Gly Asn Ile Lys Gly Gln Gly Val Cys
180 185 190

Cys Asn Glu Leu Asp Ile Trp Glu Ala Asn Ser Arg Ala Thr His Ile
195 200 205

Ala Pro His Pro Cys Ser Lys Pro Gly Leu Tyr Gly Cys Thr Gly Asp
210 215 220

Glu Cys Gly Ser Ser Gly Ile Cys Asp Lys Ala Gly Cys Gly Trp Asn
225 230 235 240

His Asn Arg Ile Asn Val Thr Asp Phe Tyr Gly Arg Gly Lys Gln Tyr
245 250 255

Lys Val Asp Ser Thr Arg Lys Phe Thr Val Thr Ser Gln Phe Val Ala
260 265 270

Asn Lys Gln Gly Asp Leu Ile Glu Leu His Arg His Tyr Ile Gln Asp
275 280 285

Asn Lys Val Ile Glu Ser Ala Val Val Asn Ile Ser Gly Pro Pro Lys
290 295 300

Ile Asn Phe Ile Asn Asp Lys Tyr Cys Ala Ala Thr Gly Ala Asn Glu
305 310 315 320

Tyr Met Arg Leu Gly Gly Thr Lys Gln Met Gly Asp Ala Met Ser Arg
325 330 335

Gly Met Val Leu Ala Met Ser Val Trp Trp Ser Glu Gly Asp Phe Met
340 345 350

Ala Trp Leu Asp Gln Gly Val Ala Gly Pro Cys Asp Ala Thr Glu Gly
355 360 365

Asp Pro Lys Asn Ile Val Lys Val Gln Pro Asn Pro Glu Val Thr Phe
370 375 380

Ser Asn Ile Arg Ile Gly Glu Ile Gly Ser Thr Ser Ser Val Lys Ala
385 390 395 400

Pro Ala Tyr Pro Gly Pro His Arg Leu
405

CLAIMS

1. A method for desizing cellulose-containing fabric or textile, wherein the fabric or textile is treated with a cellulolytic enzyme having an activity on carboxymethylcellulose (CMC) and a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} of at least 0.01 s^{-1}.

2. The method according to claim 1, wherein the catalytic activity on cellotriose at pH 8.5 corresponds to k_{cat} of at least 0.1 s^{-1}, preferably of at least 1 s^{-1}.

3. A method according to claim 1 or 2, wherein the cellulolytic enzyme is obtainable by or derived from a strain of *Humicola, Trichoderma, Myceliophthora, Penicillium, Irpex, Aspergillus, Scytalidium* or *Fusarium*.

4. A method according to claim 3, wherein the enzyme is derivable from a strain of *Humicola insolens, Fusarium oxysporum* or *Trichoderma reesei*.

5. A method according to any of the claims 1-4, wherein the enzyme is a recombinant cellulase, i.e. a cellulase essentially free from other proteins.

6. A method according to claim 5, in which the parent cellulolytic enzyme comprises the amino acid sequence of the *Humicola insolens* endoglucanase shown in SEQ ID No. 1 or an analogue of said endoglucanase which

i) is at least 60% homologous with the sequence shown in SEQ ID No. 1,

ii) reacts with an antibody raised against said endoglucanase, and/or

iii) is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding said endoglucanase.

7. A method according to claim 5, in which the parent cellulolytic enzyme comprises the amino acid sequence of the *Fusarium oxysporum* endoglucanase shown in SEQ ID No. 2 or an analogue of said endoglucanase which

i) is at least 60% homologous with the sequence shown in SEQ ID No. 2,

ii) reacts with an antibody raised against said endoglucanase, and/or

iii) is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding said endoglucanase.

8. A method according to any of the claims 1-7, in which the cellulolytic enzyme is used in an amount of corresponding to a cellulase activity between 10 and 5000 ECU per litre of desizing liquor, preferably between 50 and 500 ECU per litre of desizing liquor; preferably in an amount corresponding to 1-10 g enzyme/l, more preferably 1-5 g/l, especially 1-3 g/l.

9. A method according to any of the claims 1-8, in which the desizing treatment is performed at a temperature in the range of 30-100°C, preferably 30-60°C, and a pH in the range of 3-11, preferably 7-9.

10. A method according to any of the claims 1-9, wherein the fabric is selected from fabric made from cotton fibers, viscose (rayon) fibers, lyocell fibers, all blends of cotton, viscose or lyocell fibers with other fibers such as polyester fibers, viscose/cotton fiber blends, lyocell/cotton - fiber blends, viscose/wool fiber blends, lyocell/wool fiber blends, cotton/wool fiber blends, flax (linen), ramie, other

fabrics containing cellulose fibers, and all blends of cellulosic fibers with other fibers such as wool, polyester, polyamide and acrylic fibers.

11. A desizing composition for desizing cellulose-containing fabric comprising a cellulolytic enzyme having an activity on carboxymethylcellulose (CMC) and a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} of at least 0.01 s^{-1} and an amylolytic enzyme.

12. A desizing composition according to claim 11, wherein the cellulolytic enzyme is obtainable by or derived from a strain of *Humicola, Trichoderma, Myceliophthora, Penicillium, Irpex, Aspergillus, Scytalidium* or *Fusarium*.

13. A desizing composition according to claim 12, wherein the enzyme is derivable from a strain of *Humicola insolens*, *Fusarium oxysporum* or *Trichoderma reesei*.

14. A desizing composition according to claim 13, wherein the the parent enzyme comprises the amino acid sequence of the *Humicola insolens* endoglucanase shown in SEQ ID No. 1 or an analogue of said endoglucanase which

i) is at least 60% homologous with the sequence shown in SEQ ID No. 1,

ii) reacts with an antibody raised against said endoglucanase, and/or

iii) is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding said endoglucanase.

15. A desizing composition according to claim 13, wherein the the parent enzyme comprises the amino acid sequence of the *Fusarium oxysporum* endoglucanase shown in SEQ ID No. 2 or an analogue of said endoglucanase which

i) is at least 60% homologous with the sequence shown in SEQ ID No. 2,

ii) reacts with an antibody raised against said endoglucanase, and/or

iii) is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding said endoglucanase.

16. A desizing composition according to any of the claims 11-15, which further comprises at least one further component selected from the group consisting of wetting agents, dispersing agents, sequestering agents and emulsifying agents.

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/DK 95/00328

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 D06L1/14 C12N9/42 C12N15/56

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 D06L C11D C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 113, no. 8, 20 August 1990 Columbus, Ohio, US; abstract no. 61270, SATO HITOSHI ET AL 'Simultaneously desizing and softening fabrics' page 104; see abstract & JP,A,00 280 673 (NOVO INDUSTRI A/S) 20 March 1990 ---	1,11
A	WO,A,91 17244 (NOVO NORDISK A/S) 14 November 1991 cited in the application see figures 13,14; examples 3,4 --- -/--	1-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 1995	05.01.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Le Cornec, N

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/DK 95/00328

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,91 17243 (NOVO NORDISK A/S) 14 November 1991 cited in the application see claims see examples 2,3,7	1-7
A	CHEMICAL ABSTRACTS, vol. 111, no. 20, 13 November 1989 Columbus, Ohio, US; abstract no. 176121, TISCHLER MICHAEL ET AL 'Enzymatic pretreatment of textiles with cellulase complex preparation' page 111; see abstract & DD,A,264 947 (VEB KOMBINAT SPIRITUOSEN, WEIN UND SEKT) 15 February 1989	1,11
A	WO,A,93 20278 (NOVO NORDISK A/S) 14 October 1993 see page 5, line 8 - page 6, line 25; example 2	1
P,A	WO,A,95 02675 (NOVO NORDISK A/S) 26 January 1995 see abstract	1,3,5,6
A	WO,A,91 19794 (NOVO NORDISK A/S) 26 December 1991 see abstract see page 5; example 8	11

1

INTERNATIONAL SEARCH REPORT

...information on patent family members

Inter ...nal Application No
PCT/DK 95/00328

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9117244	14-11-91	AU-B-	652153	18-08-94
		AU-B-	7791091	27-11-91
		EP-A-	0531315	17-03-93
		US-A-	5457046	10-10-95
WO-A-9117243	14-11-91	AT-T-	118545	15-03-95
		AU-B-	639570	29-07-93
		AU-B-	7887491	27-11-91
		DE-D-	69107455	23-03-95
		DE-T-	69107455	12-10-95
		EP-A-	0531372	17-03-93
		ES-T-	2068586	16-04-95
		JP-T-	5509223	22-12-93
WO-A-9320278	14-10-93	CA-A-	2132300	14-10-93
		EP-A-	0635078	25-01-95
		JP-T-	7505451	15-06-95
WO-A-9502675	26-01-95	AU-B-	7069294	13-02-95
WO-A-9119794	26-12-91	AU-B-	8076791	07-01-92
		AU-B-	8085691	07-01-92
		WO-A-	9119807	26-12-91
		EP-A-	0576424	05-01-94